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AN EFFICIENT SYNTHESIS OF CERTAIN 5-SUBSTITUTED-2'-DEOXYURIDINE 3',5'-CYCLIC MONOPHOSPHATE P-O-ALKYL(ARALKYL) ESTERS.[1] THE CRYSTAL AND MOLECULAR STRUCTURE OF 5-IODO-2'-DEOXYURIDINE 3',5'-CYCLIC MONOPHOSPHATE P-O-METHYL ESTER WITH AXIAL METHOXY GROUP

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Abstract - Some 5-alkyl- and 5-halogeno-2'-deoxyuridine 3',5'-cyclic monophosphate P-O-alkyl(aralkyl) esters have been prepared from the silver salts of the parent cyclic monophosphates and alkyl(aralkyl) iodides. The diastereomeric phosphotriesters have been characterized by spectroscopic methods. The crystal and molecular structure of 5-iodo-2'-deoxyuridine 3',5'-cyclic monophosphate P-O-methyl ester with axial methoxy group has been determined. We failed to obtain the desired triesters from the reactions of the cAMP silver salt with alkyl iodides, contrary to previous claims in the literature.

INTRODUCTION

One of the main reasons for converting cyclic nucleotide diesters, which are fairly strong acids, into electrically neutral phosphotriesters is to obtain molecules which can penetrate much more readily the biological membranes.[3] Several synthetic methods have been proposed for this type of conversion.[4a,b;5a-g;6] One of these reactions[6] has recently been reported as unsatisfactory for obtaining phosphotriesters.[7]

Several interesting in vitro and in vivo biological effects of the phosphotriesters of natural cyclic nucleotides have been published. E.g. in vivo tumor-inhibiting properties of neutral P-O-alkyl esters of cAMP were established.[5c] However, interestingly enough cAMP ethyl ester is not able to activate either form of cAMP-dependent protein kinase (PK I and II) indicating that the charged phosphate is required for the binding of cAMP.[8]

In a previous paper[9] we described the synthesis and some biochemical properties of a series of certain 5-alkyl-2'-deoxyuridine 3',5'-cyclic monophosphates and the whole series of 5-halogeno-2'-deoxyuridine 3',5'-cyclic monophosphates. We have also reported the crystal and molecular structure of ammonium 5-fluoro-2'-deoxyuridine cyclic 3',5'-monophosphate trihydrate.[10]

Up to now only the 3',5'-cyclic phosphotriesters of the natural nucleosides have been published.[4a,b;5a-g;6] In the present paper we report a convenient synthesis (by the action of alkyl (aralkyl) iodides on the silver salts of cyclic nucleotides) and structure determination by ^{31}P, ^{13}C NMR spectroscopy and MS spectrometry of several 5-substituted-2'-deoxyuridine 3',5'-cyclic monophosphate P-O-alkyl(aralkyl) esters (1 - 9). We also present the crystal and molecular structure of 5-iodo-2'-deoxyuridine 3',5'-cyclic monophosphate P-O-methyl ester with axial methoxy group. These compounds can be regarded as electrically neutral cTMP[1] analogs. The structure-biological activity relationship among these substances will be the subject of a later communication. Contrary to earlier suggestions,[4a,b] we have found that the reactions of alkyl iodides with the cAMP silver salt occur on N1 of the base rather than on the phosphate diester group.

RESULTS AND DISCUSSION

Diastereomeric 5-substituted-2'-deoxyuridine 3',5'-cyclic monophosphate P-O-alkyl(aralkyl) esters (Table 1) have been synthesized in an S_N2 type reaction from the silver salts of the corresponding

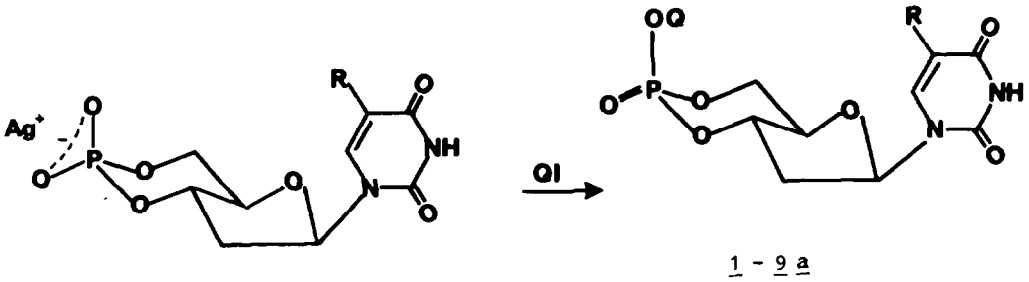
Table 1. Synthetic data for compounds 1-9

No.	Compounds	Formula	Analyses[b,c]	Yield[b] (%)	R_f(1)[d,e]
1a/1e	5-ethyl-2'-deoxyuridine 3',5'-cyclic monophosphate P-OMe	$C_{12}H_{17}N_2O_7P$	C,H,N,P	34	0.41 0.47
2a/2e	5-ethyl-2'-deoxyuridine (α) 3',5'-cyclic monophosphate P-OEt[1,2]	$C_{13}H_{19}N_2O_7P$	C,H,N,P	47	0.58 0.50
3a/3e	5-isopropyl-2'-deoxyuridine 3',5'-cyclic monophosphate P-OMe	$C_{13}H_{19}N_2O_7P$	C,H,N,P	40	0.50 0.60
4a/4e	5-isopropyl-2'-deoxyuridine 3',5'-cyclic monophosphate P-OCH_2Ph	$C_{19}H_{23}N_2O_7P$	C,H,N,P	35	0.70 0.74
5a/5e	5-butyl-2'-deoxyuridine 3',5'-cyclic monophosphate P-OMe	$C_{14}H_{21}N_2O_7P$	C,H,N,P	34	0.49 0.60
6a/6e	5-fluoro-2'-deoxyuridine 3',5'-cyclic monophosphate P-OMe	$C_{10}H_{12}FN_2O_7P$	C,H,F,N,P	49	0.20 0.23
7a/7e	5-bromo-2'-deoxyuridine 3',5'-cyclic monophosphate P-OMe	$C_{10}H_{12}BrN_2O_7P$	C,H,Br,N,P	35	0.30 0.34
8a/8e	5-iodo-2'-deoxyuridine 3',5'-cyclic monophosphate P-OMe	$C_{10}H_{12}IN_2O_7P$	C,H,I,N,P	35	0.34 0.39
9a/9e	5-iodo-2'-deoxyuridine 3',5'-cyclic monophosphate P-OCH_2Ph	$C_{16}H_{16}IN_2O_7P$	C,H,I,N,P	29	0.55 0.59

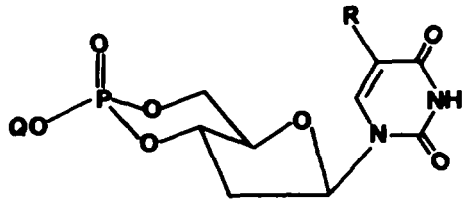
a a = axial, e = equatorial alkoxy group to the 1,3,2-dioxaphosphorinane ring
b Refer to the diastereomeric mixtures
c Found = calcd. ± 0.4%
d On 0.2 mm silica gel TLC plates
e Solvent system: $CHCl_3$:EtOH = 20:1 (v/v)

cyclic nucleotides and alkyl(aralkyl) iodides (Scheme 1). As these silver salts were readily soluble in water, we prepared them from the free acids or ammonium salts of the 3',5'-cyclic monophosphates using Amberlite IR-120 strongly acidic cation exchange resin in the Ag^+ form. Reactions of the cyclic nucleotide silver salts were performed in dry dimethylformamide (DMF) with freshly distilled alkyl(aralkyl) iodides in 5- to 25-fold molar excess at ambient temperature. No doubt silver ions promote this S_N2 type reaction enhancing the ability of the rupture of C-I bonds. Thus neither in the case of sodium salts nor in the case of ammonium salts were phosphotriesters isolated from reactions in DMF or hexamethylphosphoric triamide at ambient temperature or even at the reflux temperature of the reaction mixtures. The reactions were followed by TLC and depending on the solvent systems either one or two new spots appeared. In the latter case the diastereomers produced in 29-49% yields (Table 1) were separated. On reaction for several days followed by a simple chromatographic work-up, pure diastereomeric forms of the title compounds were obtained. The starting material could be recovered chromatographically. It is obvious that the advantage of this procedure (in case of cyclic nucleotides with thymine- or uracil-type bases) is that practically no by-products are obtained. Under the above conditions no base alkylation occured, which is one drawback of some synthetic methods published.[5f,g;16]

The diastereomeric phosphotriesters were characterized by ^{31}P and ^{13}C NMR spectroscopy. The configuration at the phosphorus atom has been determined using ^{31}P chemical shifts; phosphorus having an axial alkoxy group is more shielded and absorbs at higher field than its equatorial counterpart.[5f] This was confirmed by our X-ray study for 8a (*vide infra*). The difference in ^{31}P chemical shifts ($\delta_{axial}-\delta_{equatorial} \simeq -1.3$ ppm) (Table 2) is constant for methyl esters and increases when



	R	Q
1	$-CH_2CH_3$	$-CH_3$
2	$-CH_2CH_3$	$-CH_2CH_3$
3	$-CH(CH_3)_2$	$-CH_3$
4	$-CH(CH_3)_2$	$-CH_2C_6H_5$
5	$-(CH_2)_3CH_3$	$-CH_3$
6	$-F$	$-CH_3$
7	$-Br$	$-CH_3$
8	$-I$	$-CH_3$
9	$-I$	$-CH_2C_6H_5$



Scheme 1

changing the methyl group to ethyl in 2 (-2.4 ppm) and to benzyl in 4 and 9 (-1.6 ppm). The ratio of the diastereomers has been determined by measuring the integrated line intensities in ^{31}P spectra (Table 2). Carbon-13 spectral data (Table 2) also show a clear difference between axial and equatorial stereoisomers. The assignation of spectral lines was based on a revised assignment for the ^{13}C NMR signals of 3',5'-cyclic nucleotides as proposed recently.[11] Lines originating from axial and equatorial phosphotriesters could be assigned unambiguously from spectra of the mixtures as the diastereomeric ratio was far from 1:1 in all cases. There is a constant difference in ^{13}C chemical shifts ($\delta_{equatorial}-\delta_{axial} \approx 1.3$ ppm) and $^{2}J(P,C\alpha)$ couplings ($J_{equatorial}-J_{axial} \approx 0.8$ Hz) for α carbons. On this basis one can easily distinguish the two stereoisomers. The method is complementary to that using ^{31}P chemical shifts. Of the sugar carbons, the C3' chemical shift is the most sensitive to phosphorus configuration ($\delta_{axial}-\delta_{equatorial} = 0.9$ ppm). Smaller but significant differences were observed for C1', C2', C4' and C5' carbons. Marked differences between the two diastereomers were observed in their two-bond phosphorus-carbon couplings: $^{2}J(P,C3')$ and $^{2}J(P,C5')$ have a mean value of 5.6 and 8.6 Hz in molecules having axial alkoxy group and 3.9 and 7.3 Hz in their equatorial counterparts. Of the three-bond phosphorus-carbon couplings, only $^{3}J(P,C4')$ is sensitive to phosphorus configuration. $^{3}J(P,C2')$ is nearly constant. This latter may be due to similar cyclophosphate ring conformations in the two isomers. Examining the earlier published ^{13}C NMR chemical shifts and coupling constants for phosphorus-substituted cTMP analogs (e.g. the dimethylamidate[12] and methylphosphonate[13]) similar tendencies to those mentioned above can be noted.

The diastereomers were separated mainly by preparative silica gel TLC (for compounds 1, 2, 3, 5, 6 and 7) and in some cases by silica gel column chromatography (for compounds 4, 8 and 9), as well. Contrary to all the other cases, compound 2a has a higher R_f value on TLC than 2e (Tables 1, 2). The separated isomers have also been characterized by ^{31}P and ^{13}C NMR spectroscopy.

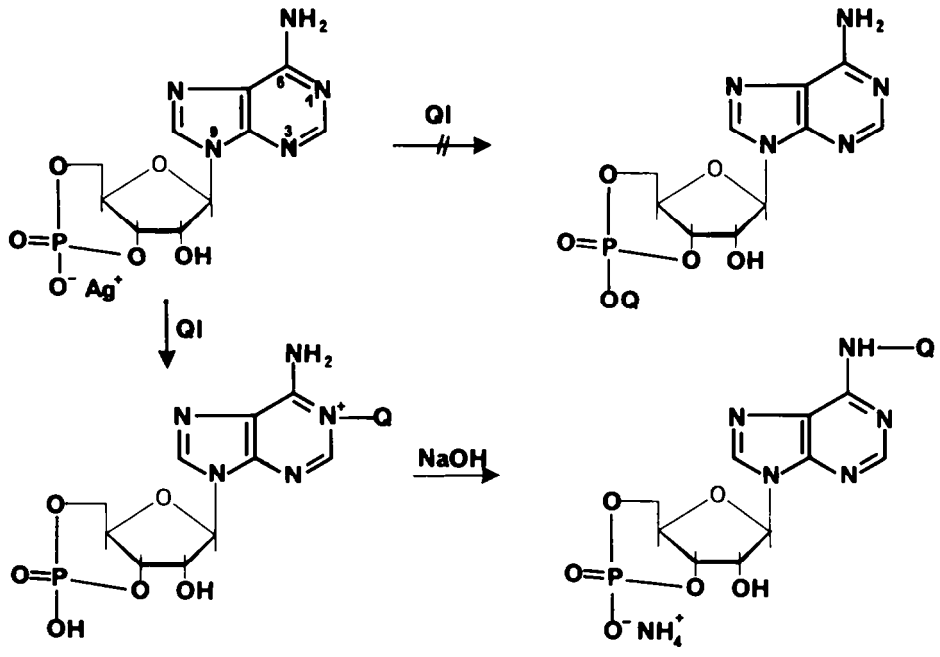
Table 2. ^{31}P and relevant ^{13}C NMR data for compounds 1-9

No.	a/e[a]	A_P[b]	R_f[c]	δ^{31}_P[d]	Carbon chemical shifts[e] $\delta_{C\alpha}$	$\delta_{C1'}$	$\delta_{C2'}$	$\delta_{C3'}$	$\delta_{C4'}$	$\delta_{C5'}$	$^n J_{P,C}$ coupling constants[f] (Hz) $^2J_{P,C\alpha}$	$^3J_{P,C2'}$	$^2J_{P,C3'}$	$^3J_{P,C4'}$	$^4J_{P,C5'}$
1a	2.4	R_P	r_f	-5.44	53.78	85.19	34.05	77.92	73.26	69.45	5.5	8.5	5.6	5.7	8.6
1e		S_P	R_f	-4.10	54.98	84.69	34.29	76.63	72.96	69.14	6.8	8.0	3.9	6.9	6.8
2a	1.5	R_P	R_f	-7.04	63.44[g]	84.86	33.57	77.62	73.03	70.01	5.5[h]	8.3	5.7	5.5	8.3
2e		S_P	r_f	-4.67	64.78[g]	85.61	33.72	76.59	72.90	69.74	6.4[h]	8.3	3.9	6.8	7.9
3a	2.0	R_P	r_f	-5.32	53.91	88.36	34.59	78.30	73.92	69.61	5.8	8.4	5.5	6.0	8.6
3e		S_P	R_f	-4.04	55.11	86.58	34.93	77.28	73.64	69.32	6.7	8.2	3.9	7.3	7.4
4a	2.4	S_P	r_f	-6.66	69.50[i]	87.59	34.80	78.11	73.84	69.60	5.4[j]	8.5	5.6	6.0	9.3
4e		R_P	R_f	-5.04	70.56[i]	86.43	35.03	77.35	73.60	69.30	5.9[j]	8.3	3.8	7.3	5.8
5a	3.1	R_P	r_f	-5.35	54.42	87.76	35.00	78.12	74.12	69.53	5.8	8.6	5.5	6.0	9.1
5e		S_P	R_f	-4.10	55.60	85.19	35.32	77.30	73.85	69.21	6.6	8.2	3.9	7.4	7.6
6a	1.8	R_P	r_f	-5.33	54.10	85.44	33.91	77.44	73.15	69.46	5.7	8.5	5.6	6.1	8.3
6e		S_P	R_f	-4.11	55.41	85.34	34.08	76.61	72.88	69.24	6.6	8.6	3.9	7.6	7.1
7a	2.2	R_P	r_f	-5.36	53.97	85.49	33.94	77.50	73.20	69.40	5.8	8.7	5.5	5.7	8.4
7e		S_P	R_f	-4.07	55.53	85.39	34.16	76.44	72.95	69.18	6.6	8.3	4.0	7.0	7.3
8a	2.3	R_P	r_f	-5.47	53.89	86.02	34.04	77.52	73.35	69.33	5.9	7.9	5.8	6.0	8.6
8e		S_P	R_f	-4.11	55.38	85.63	34.35	76.34	73.10	69.13	6.7	8.6	3.8	7.3	7.4
9a	2.3	S_P	r_f	-6.54	68.95[i]	86.27	34.45	77.64	73.54	69.10	5.5[j]	8.5	5.4	5.5	9.3
9e		R_P	R_f	-4.95	70.03[i]	85.84	34.53	76.67	73.31	69.02	6.1[j]	8.3	3.6	7.1	5.8

[a] Diastereomeric ratio (a/e)
[b] Absolute configuration of P
[c] Retention factors $r_f < R_f$ on silica gel TLC plates
[d] In ppm relative to external H_3PO_4
[e] In ppm relative to internal TMS. Data were obtained in solutions containing both members of diastereomeric pairs.
[f] $^4J(P,C1')$ couplings were not observed (resolution < 0.4 Hz).
[g] Chemical shifts of β-CH_3 carbons: 15.8 (axial), 15.87 (equatorial)
[h] For β-CH_3 carbons: $^3J(P,C\beta)_{axial} = 6.2$, $^3J(P,C\beta)_{equatorial} = 6.2$ Hz.
[i] Chemical shifts of ipso (β) carbons were not observed
[j] $^3J(P,C_{ipso})$ coupling could not be observed (see i)

The phosphotriester structure of the compounds 1 - 9 has also been confirmed by mass spectrometry. Thus, when we used a sample of 1 for MS besides the expected molecular ion (m/e 332) we could also detect the molecular ion M_2 (m/e 346). This increase (14 mass units) in mass number could be located on the base by examining the fragmentation pattern. I.e. the homologue is probably the N- or O-methyl derivative of compounds 1. As the ^{13}C NMR has shown only one methyl group (OMe) in the molecule, we repeated the MS study with a sample silylated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA). Then we observed the trimethylsilyl (TMS) derivative of 1 (Figure 1, a), and M_2 could not be detected either in unchanged or in silylated form. We conclude that M_2 is an artifact produced during vaporization.

We have recently reported[7] that the reaction of ethylene oxide with cAMP at 5 °C affords 1-(2-hydroxyethyl)-cAMP as the main product instead of the desired cAMP P-O-(2-hydroxyethyl) ester as suggested previously by Zielinski et al.[6] In order to synthesize and study the 2-hydroxyethyl phosphotriesters of cAMP we performed the reaction of cAMP silver salt (which was precipitated as a white powder on adding silver nitrate to a solution of cAMP in the requisite amount of aqueous sodium hydroxide) with 2-iodoethanol in DMF at room temperature (Scheme 2). However, we failed to detect (using silica gel TLC) and to isolate any phosphotriester from the reaction mixture. The



Q = $-CH_2CH_2OH$ 10 Q = $-CH_2CH_2OH$ 11
$-CH_3$ 12 $-CH_3$ 13

Scheme 2

main product obtained proved to be identical in every respect (chromatographic, analytical, spectroscopic) with that isolated in the reaction of ethylene oxide with cAMP[7], 1-(2-hydroxyethyl)-cAMP, 10 (Table 3). To support our suspicion that the reactions of alkyl iodides (e.g. methyl iodide) with cAMP silver salt do not produce phosphotriesters, as proposed earlier,[4a,b] but instead give 1-methyl-cAMP, we have also performed that reaction. Our attempts to detect phosphotriesters in the reaction mixture by silica gel TLC and to isolate them from the reaction products proved unsuccessful in this case, too. In the proton decoupled carbon-13 spectrum of the major product, isolated by DEAE Sephadex column chromatography, the methyl signal appears at 38.75 ppm representing an N^+-methyl group instead of 53-55 ppm as would be expected for the methyl triester and fails to show any coupling with phosphorus. The site of methyl substitution, on the other hand, could be readily identified by recording the proton decoupled ^{13}C spectrum (in D_2O) of the compound (N^6-methyl-cAMP.NH_3, 13) obtained via Dimroth rearrangement[14] on alkaline treatment of the reaction product (1-methyl-cAMP, 12) (Table 4 and Scheme 2). The changes in the anion exchange column and silica gel thin layer chromatographic properties of these compounds and in the ^{13}C chemical shifts of the methyl group (a 10 ppm decrease for 13 relative to that in 12) were very similar to those of 10 and 11 (Tables 3, 4).

Further confirmation of the above structure was presented by mass spectrometry as the molecular ion (m/e 487) corresponding to the bis-trimethylsilyl derivative of 1-methyl-cAMP (Figure 1, b) and several other characteristic fragments (e.g. M-base, m/e 339; base + H, m/e 149) could be detected in the MS spectrum of the sample silylated with BSTFA.

Our findings concerning the alkylation of cyclic nucleotides under the conditions used can be summarized as follows: the relative order of the reactivity of the cyclic mononucleotide bases with ethylene oxide is adenine>thymine≈uracil (slow reaction), and with alkyl iodides is adenine >> thymine≈uracil (practically no reaction).[15]

Attempts to prepare triesters of nucleoside 3',5'-cyclic monophosphates by alkylation routes using alkyl iodides have been reported previously. The potassium salt of adenosine 3',5'-cyclic phosphate was esterified (room temperature, 20 hr) by methyl iodide in dimethyl sulphoxide (DMSO)

Table 3. Synthetic data for compounds 10, 12 and 13

No.	Compounds	Formula	Analyses[a]	Yield (%)	$R_f^b(2)^c$
	cAMP[d]				0.55
10	1-(2-hydroxyethyl)-cAMP	$C_{12}H_{16}N_5O_7P$	C,H,N,P	41	0.38
11	N^6-(2-hydroxyethyl)-cAMP.NH_3[d]				0.49
12	1-methyl-cAMP	$C_{11}H_{14}N_5O_6P$	C,H,N,P	58	0.34
13	N^6-methyl-cAMP.NH_3	$C_{11}H_{17}N_6O_6P$	C,H,N,P	47	0.50

[a] Found = calcd. ± 0.4%

[b] On 0.2 mm silica gel TLC plates

[c] Solvent system: isobutiric acid: 25% ammonium hydroxide:water = 66:1:33 (v/v)

[d] Compounds for comparison

Table 4. Relevant carbon-13 data[a] for compounds 10-13

No.	base substituents		sugar carbons								
	$\delta_{C\alpha}$	$\delta_{C\beta}$	$\delta_{C1'}$	$\delta_{C2'}$	$\delta_{C3'}$	$\delta_{C4'}$	$\delta_{C5'}$	$^3J_{(P,C2')}$	$^2J_{(P,C3')}$	$^3J_{(P,C4')}$	$^2J_{(P,C5')}$
10	53.93	59.29	93.52	73.57	78.04	73.33	68.19	8.3	4.4	4.4	7.0
11	43.70	60.98	92.28	73.06	77.98	72.47	68.05	7.7	4.6	3.7	7.3
12	38.75		93.51	73.57	78.06	73.32	68.22	8.1	4.5	4.5	7.1
13	28.22		92.12	73.00	77.89	72.46	67.99	7.8	4.4	3.8	7.2

[a] Measured in D_2O. Chemical shifts are in ppm, coupling constants in Hz.

in the presence of one equivalent of 18-crown-6. The resulting diastereomeric adenosine 3',5'-cyclic phosphate methyl esters, however, also were methylated on the adenine ring, presumably at N1 (not isolated, used in solution).[16] By contrast, only 1-methyladenosine cyclic 3',5'-phosphate (isolated, yield 31%) was formed from the 1,5-diazabicyclo[5.4.0]-5-undecene (DBU) salt of cAMP and methyl iodide in DMSO (ambient temperature, 2 Hr).[17]

X-Ray determination of the molecular structure of 8a

As shown by a perspective view of structure 8a (Figure 2) computed from the final fractional atomic coordinates given in Tables 5 and 6, the P-methoxy group, in accord with the spectroscopic evidence (vide supra) is axial to the dioxaphosphorinane ring. As expected[18] the methoxy group does not influence the mean P-O distance of 1.543(6) Å which agrees well with that (1.547(1) Å) found e.g. in 5-fluoro-2'-deoxyuridine cyclic 3',5'-monophosphate anion (hereinafter: 5-F-cdUMP).[10] The puckering parameters[19]: $Q = 0.56$ Å, $\varphi = 1.0^\circ$, $\theta = 157.7^\circ$ of this phosphorinane ring indicate a distorted chair conformation (shifted slightly towards an envelope like form) as has been found with other cyclic nucleotides. The almost perfect half-chair conformation of the ribofuranose ring with a C_2 axis bisecting C(1') observed in 5-F-cdUMP now is slightly destroyed, the present lowest asymmetry factors[20] are $fC_2 = 4.7$ pm ($fC_s = 7.4$ pm). There is, however, a markedly altered rotation about the glycosidic N(1)-C(1') bond. While in 5-F-cdUMP $\chi = 50.3^\circ$ (anti) now it falls in the syn range (-98.1°). The pyrimidine base is fairly planar while iodine is 0.102 Å out of its best plane ($-0.02806X + 0.94853Y - 0.31543Z = 4.29447$). Although the electronegativity of I is significantly lower than that of F it has the same influence upon the C(4)-C(5)-C(6) bond angle as fluorine atom. Since molecule 8a has only one active H atom bound to N(3), in the crystal lattice there is only one hydrogen bond: N(3)-H(3)...O(6) [1/2-x,-y,1/2+z] N...O = 2.77(1), H...O = 1.86(1) Å, ∡NH...O = 158.2(10)°.

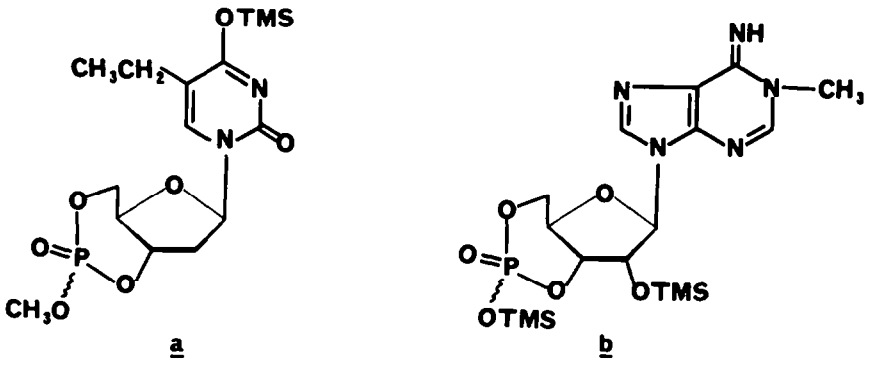


Figure 1

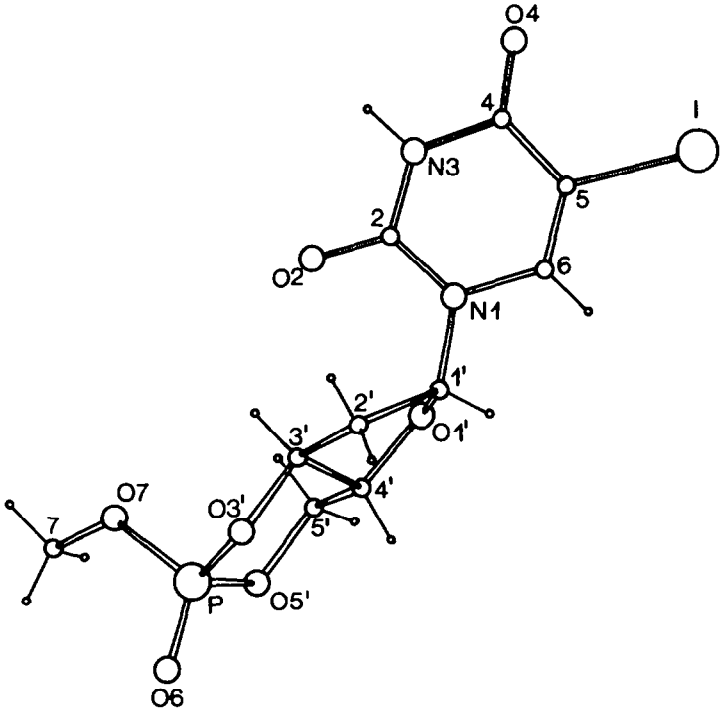


Figure 2
A perspective view of molecule 8a with atomic numbering. Atoms are carbon unless indicated otherwise. The H atoms are shown but not labelled.

EXPERIMENTAL

Materials. Parent cyclic nucleotides were synthesized as described previously.[9] 2-iodoethanol was obtained from 2-bromo- or 2-chloroethanol on refluxing with NaI in acetone or methanol. Benzyl iodide was synthesized in a similar way. Pre-coated silica gel TLC plates (DC-Alufolien, Kieselgel 60 F_{254}, 0.2 mm x 20 cm x 20 cm and DC-Fertigplatten, Kieselgel 60 F_{254}, 0.25 mm x 20 cm x 20 cm) were purchased from Merck, Darmstadt. Silica gel (Kieselgel 40, 0.063-0.200 mm) used for column chromatography was the product of Merck, Darmstadt. Amberlite IR-120 (H^+) was purchased from Fluka AG, Switzerland. DEAE Sephadex A-25 (Cl^-) was the product of Pharmacia Fine Chemicals, Sweden.

Table 5. Fractional coordinates ($\times 10^4$) and mean temperature factors for non-hydrogen atoms. E.s.d.'s are given in parentheses, $B_{eq} = 4[B_{11}B_{22}B_{33}/(a^{*2}b^{*2}c^{*2})]^{1/3}$

Atoms	x/a	y/b	z/c	B_{eq} (Å^2)	Atoms	x/a	y/b	z/c	B_{eq} (Å^2)
I	-1029(1)	9090(1)	9615(1)	3.5(1)	O(1')	4365(5)	11492(5)	11109(3)	3.1(2)
P	8633(2)	12090(2)	12286(1)	3.0(1)	C(2')	5445(9)	9662(8)	11797(5)	3.1(3)
N(1)	3463(7)	9574(6)	10602(4)	2.9(2)	C(3')	6559(8)	10695(7)	11645(4)	2.5(3)
C(2)	4370(8)	9280(7)	9916(5)	2.8(3)	C(4')	5581(8)	11862(7)	11645(4)	2.5(3)
N(3)	3721(6)	8873(6)	9176(4)	2.9(2)	O(3')	7681(6)	10826(5)	12311(3)	3.2(2)
C(4)	2173(8)	8785(7)	9019(5)	2.7(3)	C(5')	6429(9)	12992(7)	11330(5)	3.1(3)
C(5)	1286(6)	9123(7)	9755(5)	2.7(3)	O(5')	7571(6)	13212(5)	11986(4)	3.3(2)
C(6)	1905(8)	9486(7)	10498(5)	2.8(3)	O(6)	9315(6)	12405(6)	13104(4)	4.3(3)
O(2)	5731(6)	9400(6)	9977(4)	4.3(3)	O(7)	9740(7)	11856(6)	11525(4)	4.4(3)
O(4)	1742(7)	8456(6)	8314(4)	4.0(3)	C(7)	10839(12)	12815(12)	11360(8)	6.7(5)
C(1')	4000(8)	10205(7)	11354(5)	2.9(3)					

Table 6. Fractional coordinates ($\times 10^3$) for hydrogen atoms

Atoms	x/a	y/b	z/c	B_i (Å^2)		x/a	y/b	z/c	B_i (Å^2)		x/a	y/b	z/c	B_i (Å^2)
H(3)	437	862	872	5.0	H(2'B)	530	953	1240	5.0	H(3')	712	1055	1113	5.0
H(6)	127	969	1097	5.0	H(5'A)	687	1282	1078	5.0	H(7A)	1146	1260	1088	7.9
H(1')	322	1010	1177	5.0	H(5'B)	579	1371	1128	5.0	H(7B)	1036	1363	1121	7.9
H(2'A)	576	889	1154	5.0	H(4')	524	1213	1219	5.0	H(7C)	1146	1298	1184	7.9

Methods. ^{31}P NMR spectra were run in DMSO-d_6 on a Varian FT-80A NMR spectrometer operating at 32.2 MHz. ^{31}P chemical shifts upfield from external 85% H_3PO_4 are negative. No susceptibility corrections were made. ^{13}C NMR spectra were recorded in $CDCl_3$-DMSO-d_6 solvent mixture on a disk-augmented Varian XL-100/15 spectrometer operating at 25.2 MHz. TMS was used as the internal reference.

Mass spectrometric measurements were carried out on an AEI MS-902 double focussing instrument with ionizing energy 70 eV and an ion source temperature of 200 °C. All samples were introduced by direct probe. Solvent systems used for thin layer and silica gel column chromatography were chloroform:ethanol = 20:1 (system 1) and isobutyric acid:25% ammonium hydroxide:water = 66:1:33 (system 2) (v/v). DEAE Sephadex column chromatographic separations were performed with the help of a Spectromom 195 spectrophotometer (MOM, Hungary) equipped with flow-through cells (Starna Ltd., England) and a potentiometric recorder (Type OH 814/1, Radelkis, Hungary).

General Procedure for the Synthesis of 5-substituted-2'-deoxyuridine 3',5'-cyclic Monophosphate P-O-alkyl(aralkyl) Esters, 1-9

The free acid or ammonium salt of the 5-substituted-2'-deoxyuridine 3',5'-cyclic monophosphate was dissolved in water and the solution was applied to a column of Amberlite IR-120 (Ag^+). The column then was washed with water. The UV-absorbing (260 nm) fractions were evaporated and dried over P_2O_5 in vacuo and darkness.

To a suspension of 1 mmol of 5-substituted-2'-deoxyuridine 3',5'-cyclic monophosphate silver salt in 5 ml of dry DMF, 25 mmol of distilled alkyl iodide was added at ambient temperature. Only 5 mmol was used in the case of benzyl iodide. After several hours complete dissolution occured. The solution was left for about 3 days in darkness. Then the alkyl iodide was removed (if possible) by evaporation and approx. 30 ml of $CHCl_3$-EtOH (1:1) was added to the solution. The precipitated solid was filtered off, and the filtrate was evaporated to an oily residue which was then taken up in a small volume of $CHCl_3$-EtOH (20:1) and applied to a silica gel column (2.5x40 cm; eluted with $CHCl_3$-EtOH, 20:1). The appropriate fractions were evaporated to dryness or to a small volume and the diastereomeric phosphotriesters were precipitated by ether.

Chromatographic separations of the diastereomeric phosphotriesters were mainly performed on pre-coated TLC plates (DC-Fertigplatten, Kieselgel 60 F_{254}, 0.25 mm x 20 cm x 20 cm). The developing system in every case was $CHCl_3$:EtOH = 20:1. To obtain satisfactory separations, the plates were developed 2 to 3 times in the same solvent system. The separated diastereomers were extracted from silica gel with $CHCl_3$:EtOH = 1:1 solvent mixture.

Reactions of alkyl iodides with cAMP silver salt

cAMP.Ag. To a suspension of 1 mmol of cAMP in 3 ml of water 1 ml of 1 M aqueous sodium hydroxide was added; then 1.1 mmol of silver nitrate in 3ml of water was poured into this solution. The white precipitate was filtered off and washed thoroughly with water and dried over P_2O_5 in vacuo and darkness.

1-alkyl-cAMPs, 10, 12. The reactions of methyl iodide and 2-iodoethanol with the cAMP silver salt were performed in the same manner as described for the 5-substituted-2'-deoxyuridine 3',5'-cyclic phosphate silver salts except that the reaction mixture was poured into water, the precipitated solid was filtered off, and the filtrate was applied to a DEAE Sephadex A-25 (HCO_3^-) column. The 1-alkyl-cAMP derivatives could be eluted with water from this column.

N^6-methyl-cAMP.NH_3, 13. The pH of a solution of 1-methyl-cAMP in water was adjusted to approximately 10 with 2 M aqueous sodium hydroxide and left for 3 days at room temperature. Then the solution was applied to a DEAE Sephadex A-25 (HCO_3^-) column and chromatographed using a linear gradient of water and ammonium bicarbonate (1 M).

Crystal structure of 5-iodo-2'-deoxyuridine 3',5'-cyclic monophosphate P-O-methyl ester (8a)

Crystal data: $C_{10}H_{12}PIN_2O_7$, MW = 430.10, orthorhombic, a = 8.956(2), b = 10.520(1), c = 15.496(2) Å, U = 1460.0(6) $Å^3$, D_c = 1.957 $g.cm^{-3}$, Z = 4, F(000) = 840, space group $P2_12_12_1$ (from systematic absences). Intensities of 2451 independent reflections were collected in the range $1.5<\theta<25^\circ$ by an ω-2θ scan on an Enraf-Nonius CAD-4 diffractometer with graphite monochromated MoK$_\alpha$ (λ = 0.71073 Å) radiation. Cell constants were determined by least squares refinement from the setting angles of 25 reflections. After data reduction, 1941 reflections with $I-3\sigma(I)>0$ were taken as observed. No absorption correction was applied (μ = 23.1 cm^{-1}). The fractional coordinates of I atom were determined by Patterson method. The iodine contribution alone gave R = 0.30. After two successive cycles of isotropic refinement of these iodine coordinates (R = 0.24) a Fourier synthesis gave the positions of all non-hydrogen atoms (R = 0.17). The structure was refined by full-matrix least-squares method anisotropically to R = 0.064. At this stage the H positions were generated from assumed geometries but were not refined. After the assignment of the correct enantiomeric form R was improved to a final 0.043 (R = 0.052). Scattering factors were taken from standard tables[21]. Bond distances and angles and relevant torsion angles are given in Tables 7 and 8.

All calculations were performed on a PDP11/34 minicomputer with the use of the SDP-34 system of Enraf-Nonius with local modifications.

Table 7. Interatomic distances (Å) and bond angles (°) with their e.s.d.'s in parentheses

Atoms	Lengths	Atoms	Lengths	Atoms	Lengths	Atoms	Lengths
I -C(5)	2.085(5)	O(3')-C(3')	1.447(8)	C(2')-C(1')	1.572(11)	C(5)-C(4)	1.435(10)
P -O(6)	1.446(6)	C(3')-C(4')	1.508(10)	C(1')-O(1')	1.444(9)	C(4)-N(3)	1.411(9)
P -O(7)	1.560(6)	C(3')-C(2')	1.494(11)	C(1')-N(1)	1.425(10)	C(4)-O(4)	1.209(10)
P -O(3')	1.580(6)	C(4')-C(5')	1.493(10)	N(1) -C(6)	1.408(10)	N(3)-C(2)	1.355(10)
P -O(5')	1.586(6)	C(4')-O(1')	1.424(8)	N(1) -C(2)	1.373(10)	C(2)-O(2)	1.229(9)
O(7)-C(7)	1.432(13)	C(5')-O(5')	1.460(10)	C(6) -C(5)	1.334(11)		

Atoms	Angles	Atoms	Angles	Atoms	Angles
O(6) -P -O(7)	115.5(6)	C(3')-C(4')-O(1')	102.8(9)	N(1)-C(6)-C(5)	122.0(12)
O(6) -P -O(3')	113.5(6)	C(5')-C(4')-O(1')	114.6(10)	I -C(5)-C(6)	120.5(9)
O(6) -P -O(5')	109.9(6)	C(4')-C(5')-O(5')	104.8(10)	I -C(5)-C(4)	117.6(9)
O(7) -P -O(3')	103.2(5)	P -O(5')-C(5')	120.4(8)	C(6)-C(5)-C(4)	121.8(11)
O(7) -P -O(5')	106.1(6)	C(3')-C(2')-C(1')	102.5(10)	C(5)-C(4)-N(3)	113.0(11)
O(3')-P -O(5')	108.1(5)	C(2')-C(1')-O(1')	105.6(10)	C(5)-C(4)-O(4)	127.8(12)
P -O(7) -C(7)	117.4(11)	C(2')-C(1')-N(1)	117.8(11)	N(3)-C(4)-O(4)	119.2(12)
P -O(3')-C(3')	115.9(7)	O(1')-C(1')-N(1)	107.4(10)	C(4)-N(3)-C(2)	126.0(11)
O(3')-C(3')-C(4')	109.0(10)	C(4')-O(1')-C(1')	106.0(9)	N(1)-C(2)-N(3)	118.2(11)
O(3')-C(3')-C(2')	114.9(10)	C(1')-N(1) -C(6)	117.3(11)	N(1)-C(2)-O(2)	120.3(12)
C(4')-C(3')-C(2')	101.8(10)	C(1')-N(1) -C(2)	122.6(11)	N(3)-C(2)-O(2)	121.5(12)
C(3')-C(4')-C(5')	110.7(10)	C(6) -N(1) -C(2)	118.9(11)		

Table 8. Relevant torsion angles (°) with their e.s.d.'s in parentheses

χ	O(1')-C(1')-N(1) -C(6)	-98.1(11)	ψ'	O(3')-C(3')-C(4')-C(5')	69.4(10)
	O(1')-C(1')-N(1) -C(2)	69.1(12)	ψ	C(3')-C(4')-C(5')-O(5')	-66.0(10)
	C(3')-C(2')-C(1')-N(1)	112.8(12)		P -O(5')-C(5')-C(4')	52.1(8)
τ_0	C(4')-O(1')-C(1')-C(2')	-21.8(9)	ω	C(5')-O(5')-P -O(3')	-37.4(9)
τ_1	O(1')-C(1')-C(2')-C(3')	-7.0(9)		O(5')-C(5')-C(4')-O(1')	178.3(12)
τ_2	C(1')-C(2')-C(3')-C(4')	31.1(9)		O(6) -P -O(3')-C(3')	158.3(10)
τ_3	C(2')-C(3')-C(4')-O(1')	-46.0(9)		O(7) -P -O(3')-C(3')	-75.9(9)
τ_4	C(3')-C(4')-O(1')-C(1')	42.3(9)		C(7) -O(7) -P -O(6)	-52.0(12)
	C(5')-C(4')-O(1')-C(1')	162.4(10)		C(7) -O(7) -P -O(5')	70.0(11)
ω'	O(5')-P -O(3')-C(3')	36.1(7)			
φ'	P -O(3')-C(3')-C(4')	-52.8(8)			

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REFERENCES AND NOTES

[1] Abbreviations: 5-substituted-2'-deoxyuridine 3',5'-cyclic monophosphate P-O-alkyl ester = 1-(2-deoxy-ß-D-ribofuranosyl)-5-substituted-uracil 3',5'-cyclic monophosphate P-O-alkyl ester, cAMP 9-ß-D-ribofuranosyladenine 3',5'-cyclic phosphate, cTMP = 1-(2-deoxy-ß-D-ribofuranosyl)-thymine 3',5'-cyclic phosphate, 5-ethyl-2'-deoxyuridine (α) 3',5'-cyclic monophosphate P-OEt = 1-(2-deoxy-α-D-ribofuranosyl)-5-ethyluracil 3',5'-cyclic monophosphate P-O-ethyl ester.
[2] Two compounds (2a and 2e) with α-glycosidic configuration have also been prepared.
[3] T.Posternak, 'Advances in the Biosciences', eds. by G.Cehovic and G.A.Robison, Pergamon Press: Oxford, 1979, Vol. 24, p. 1-13.
[4] (a) J.-G.Falbriard, T.Posternak and E.W.Sutherland, Biochim.Biophys.Acta, 148, 99 (1967), and ref. 1 therein, (b) W.Xu and Z.Liho, Acta Pharmaceutica Sinica, 15, 396 (1980).
[5] (a) G.Baschang and V.Kvita, Angew.Chem., 85, 44 (1973), (b) J.H. van Boom, P.M.J.Burgers, P. van Deursen and C.B.Reese, J.Chem.Soc., Chem.Commun., 618 (1974), (c) F.A.Cotton, R.G.Gillen, R.N.Gohil, E.E.Hazen, Jr., C.R.Kirchner, J.Nagyvary, J.P.Rouse, A.G.Stanislowski, J.D.Stevens and P.W.Tucker, Proc.Natl.Acad.Sci. USA, 72, 1335 (1975) and refs. 4 and 5 therein, (d) J.Engels and R.Reidys, Experientia, 34, 14 (1978), (f) J.Engels, Bioorg. Chem., 8, 9 (1979), (g) K.K. Ogilvie, S.L.Beaucage, M.F.Gillen and D.W.Entwistle, Nucleic Acids Res., 6, 2261 (1979) and ref. 20 therein.
[6] W.S.Zielinski, S.Hynie and J.Smrt, Coll.Czech.Chem.Commun., 43, 1655 (1978).
[7] J.Bêres, E.Moravcsik and L.Radics, Heterocycles, 16, 1951 (1981).
[8] J.P.Miller, 'Advances in Cyclic Nucleotide Research', eds. by J.E.Dumont, P.Greengard and G.A.Robison, Raven Press: New York, 1981, Vol. 14, p. 335-344.
[9] Manuscript in preparation for J.Med.Chem.
[10] T.Koritsánszky, A.Kálmán, J.Bêres, K.Kömives and L.Ötvös, Acta Cryst., C39, 772 (1983).
[11] M.Kainosho, Org.Magn.Reson., 12, 548 (1979) and refs. 1 and 2 therein.
[12] W.J.Stec and W.S.Zielinski, Tetrahedron Letters, 1361 (1980).
[13] G.S.Bajwa and W.G.Bentrude, Tetrahedron Letters, 4683 (1980).
[14] J.D.Engel, Biochem.Biophys.Res.Commun., 64, 581 (1975).
[15] B.Singer, 'Progress in Nucleic Acid Research and Molecular Biology', ed. by W.E.Cohn, Acad.Press: New York, 1975, Vol. 15, p. 219-284.
[16] R.L.Jarvest, G.Lowe and B.V.L.Potter, J.Chem.Soc., Perkin Trans. 1, 3186 (1981).
[17] R.B.Meyer, Jr., D.A. Shuman, R.K.Robins, J.P.Miller and L.N.Simon, J.Med.Chem., 16, 1319 (1973).
[18] A.Kálmán, J.Chem.Soc. (A) 1759 (1971).
[19] D.Cremer and J.A.Pople, J.Am.Chem.Soc., 97, 1345 (1975).
[20] A.Kálmán, M.Czugler and K.Simon, Struct. and Biol.Activity (Eds. by J.F.Griffin and W.L.Duax) p. 367-376. Elsevier Biomedical, New York (1982).
[21] International Tables for X-ray Crystallography, Vol. III. Kynoch Press, Birmingham (1962).